



RAPD Analysis of Genetic Variation in a Rare Island Endemic Tree Species, *Colubrina oppositifolia*

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AUG 15 1995

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Colubrina oppositifolia is a rare, dry forest tree endemic to the Hawaiian Islands. *C. oppositifolia*, or *kauila*, was known and treasured for its beautiful hard wood. Kauila are 5-13 m tall trees characterized by a distinctive bark. It occurs in dry to mesic forest, 240-920 m in elevation and is best known on northwestern O'ahu and leeward Hawai'i (Wagner et al. 1990). Joseph Rock (1913), one of the pioneering Hawaiian botanists, expressed wonder at the diversity of tree species of the dry forest. However, today under the threat of continual degradation of this habitat by fire, alien plant species, cattle grazing, goat browsing, and commercial land development only a few fragmented populations of this taxa remain. Consequently, kauila recently received federal

Published by the Hawaiian Botanical Society, which was founded in 1924 to "advance the science of botany in all its applications, encourage research in botany in all its phases, promote the welfare of its members and develop the spirit of good fellowship and cooperation among them." Any person interested in the plant life of the Hawaiian Islands is eligible for membership. Information may be obtained from the Society:

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status and was listed as an endangered species (Mehrhoff 1994).

Multipurpose trees have recently received increased attention because of the focus of local, national, and international organizations on peoples from rural communities (Simons 1991). Multipurpose (syn. non-industrial) trees provide a wide range of products including fuelwood, fodder, green manure, building materials, as well as environmental benefits such as erosion control and habitat for native species (Simons 1991). Kauila was utilized in all aspects of the Hawaiian economy (Krauss 1993). The hard wood qualities of kauila allowed it to replace the role of metal in the Hawaiian economy and be used for *o'ō* (digging stick) the primary tool for cultivation, fishing spears, a weaving apparatus, a scraping board for *kapa* (cloth) preparation, weapons such as daggers and javelin, runners on sleds, and handles for war clubs. The beautiful red hue of kauila heartwood was crafted to make musical instruments, *kā lā 'au* (beating sticks) used to keep rhythm in traditional *hula* (dance) and for *'ūkēkē* (musical bow). For

many of these objects kauila was the primary source or if not, the most preferred.

Unfortunately, like most multipurpose trees, the basic biology (breeding system, phonology, reproductive biology) of kauila is also poorly understood. Similarly, little is known about the gene pool and the structure of variation within kauila.

This study attempts to assess the amount and structure of genetic variation that is present in kauila. This information can be applied to determine the status and genetic health of the fragmented populations. One of the consequences of a population becoming small or fragmented is the increase in the frequency of inbreeding. As population numbers dwindle, there are fewer and fewer individuals with which to cross pollinate. Variant forms of a plant that would be maintained in a large population size may not have the opportunity to reproduce under conditions of stressed population size. The result will be the loss of genetic variation.

A technique called RAPDs (random

amplified polymorphic DNA) will be used to assess genetic variation. RAPDs utilizes random 10-base pair oligonucleotides that prime PCR amplification over the genome of an organism. RAPDs have been increasingly employed to assess variation because of the availability of several hundred commercially available primers that produce a large number of scorable loci, the ability to detect high levels of polymorphism and unique genetic patterns, and the lack of need for prior sequence information for PCR amplification (Williams et al. 1990).

Materials and Methods

Plant material and collection

Colubrina oppositifolia is known to occur in dry to mesic forest at elevations of 240-920 m in the Hawaiian archipelago (Wagner et al. 1990). It tends to prefer well-drained west facing slopes that are often steeply inclined. Two native tree species, *Diospyros* sp. (*Mama*) and the more rare *Caesalpinia kavaïense* (*uhiuhi*) are often associated with kauila in its native habitat. However, in disturbed areas, two

alien tree species, *Psidium cattleianum* (strawberry guava) and *Schinus terebinthifolius* (Christmas berry), often dominate its habitat.

Three considerably large populations make up the best known examples of extant members of this taxa. Two populations have been documented in leeward Hawai'i (Kaūpūlehu, North Kona and Kapu'a Farms, South Kona) and the third is found in the northern Wai'anae Mountains (East Makaleha Valley) on the island of O'ahu (Mehrhoff 1994).

The South Kona and East Makaleha Valley populations were chosen for this study due to accessibility and the availability of total DNA already extracted. Fresh leaf material was collected from 10-15 selected trees from each population. The trees selected possessed a minimum diameter at breast height (dbh) of 10 cm, were 4 m in height or taller, and covered an area of approximately 4 acres. Personal observations estimate the East Makaleha Valley population to contain 30-40 trees greater than or equal to 3 m in height which covered an area of approximately 4 acres. The South

Table 1. Primers used to assess variation in *Colubrina oppositifolia*.

Primer	Sequence	# of RAPD fragments
OP A2	5' TGCCGAGCTG	3
OP A11	5' CAATCGCCGT	5
OP B1	5' GTTTCGCTCC	4
OP B8	5' AGGGAACGAG	3
OP B17	5' GTCCACACGG	2

units *Taq* Polymerase (Promega). Each reaction was overlaid with 50 µl mineral oil.

Amplifications were performed in a Hybaid Omni Gene thermocycler pro-

Kona population contains the same number and density of trees (Morden personal communication).

DNA extraction

Total DNA was extracted from 0.5-1.0 g of fresh leaf material using the CTAB (cetyltrimmonium bromide) method of Doyle and Doyle (1987) and purified on cesium chloride-ethidium bromide gradients. Water saturated butanol was used to remove the ethidium bromide and the banded DNA dialyzed to remove the cesium.

RAPD-PCR Amplification

Amplifications were carried out in 25 µl reaction volumes of 25 ng of genomic DNA, 2 µM random Homer Operon primers, and 200 µM each of dATP, dCTP, dGTP, and dTTP, 1X *Taq* Polymerase PCR Buffer, 1.5 mM MgCl₂, and 4.0

grammed for one cycle at 94 C for 3 min. 35 C for 30 sec. and 72 C for 2 min. followed by 43 cycles at 95 C for 45 sec. 35 C for 30 sec. and 72 C for 2 min. and a final cycle at 94 C for 45 sec. 35 C for 30 sec. and 72 C for 6 min. Amplification products were mixed with loading dye (20 mM EDTA, 10% glycerol, 1% sarcosyl w/ bromophenol blue and xylene cyanol) and separated in 1.5% agarose in 0.5 X TBE (tris-borate-EDTA) buffer with 125 ng ethidium bromide per liter. Sizes of the amplification products were estimated by using a marker produced from pBS plasmid (Stratagene, La Jolla, CA) digested with restriction enzymes to produce fragments in a size range of 0.448 - 2.96 kb. Gels were run for 2-3 hours at 72 volts and photographed on a UV transilluminator.

Results

Fifteen 10-base pair primers were evaluated for their ability to prime PCR amplification of *C.*

oppositifolia genomic DNA. Primers were chosen based on their ability to successfully amplify according to the rating system of

Fritsch et al. (1993). In a preliminary survey, the total DNA of three East Makaleha Valley individuals were used as templates to screen primers. Twelve of the fifteen primers produced bands that were visualized on agarose gels: OPA1, OPA2, OPA3, OPA4, OPA11,

Table 2. RAPD data of two populations of *Colubrina oppositifolia* for five random oligonucleotide primers. The top half of the table represents individuals from Makaleha Valley, O'ahu and the bottom half represents individuals from South Kona, Hawai'i. A zero(0) indicates the absence of a band and a one (1) indicates the presence of a band.

	A2			A11			B1			B8			B17		
Individual															
J1	1	1	1	1	0	0	0	1	1	1	1	1	1	0	0
J2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
J4	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1
J5	1	1	1	1	0	1	1	0	0	0	0	0	1	1	1
J6	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1
J7	1	1	1	1	0	1	1	0	1	1	1	1	0	1	1
5	1	1	1	1	0	1	1	1	1	1	1	0	0	0	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
12	1	0	0	1	1	1	1	0	1	1	1	0	1	1	1
13	1	1	1	1	0	0	1	0	0	0	0	0	1	1	1
1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1
2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
3	1	1	1	0	0	0	0	0	1	0	1	0	0	0	0
4	1	1	1	1	0	0	1	1	0	0	0	0	0	1	1
5	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1
6	1	1	1	1	0	1	1	1	0	0	0	0	1	1	1
7	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1
8	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1
9	1	1	1	1	0	0	0	0	1	1	1	1	1	0	1
10	1	1	1	1	0	0	0	0	1	1	1	1	0	1	1

Table 3. Estimates of genetic diversity within population for *C. oppositifolia* using Shannon's H_o values calculated from RAPD data from five random oligonucleotide primers.

Primer	Population	
	Makaleha Valley	South Kona
OP A2	0.940	0.000
OP A11	1.696	1.557
OP B1	0.802	1.168
OP B8	1.089	1.359
OP B17	0.500	0.500
Average	1.005	0.917

quency. A phenotype is the RAPD profile of a given primer for an individual. The phenotype of Makaleha Valley - J1 for primer OPA2 is 111. However, Makaleha Valley - J4 has phenotype 110 for

OPB1, OPB7, OPB8, OPB13, OPB17, OPB20, and OPC8. Five primers produced distinguishable bands and were used to score individuals (Table 1). Three primers failed to amplify the genomic DNA: OPA5, OPB4, and OPC2.

Both populations exhibited high levels of polymorphism. The Makaleha Valley individuals were polymorphic at 16 of 17 loci (94%). South Kona individuals were polymorphic at 13 of the 17 loci (76%). Table 2 presents the data matrix.

Estimates of genetic diversity were obtained using Shannon's index of $H_o = -\sum p_i \ln p_i$ (Lewontin 1973; King and Schaal 1989; Chalmers et al. 1992; Dawson et al. 1995), where p_i is the phenotypic fre-

quency. A phenotype is the RAPD profile of a given primer for an individual. The phenotype of Makaleha Valley - J1 for primer OPA2 is 111. However, Makaleha Valley - J4 has phenotype 110 for primer OPA2. Estimates of genetic diversity within populations are given in Table 3. Overall, both populations exhibit similar levels of within population variability (Table 3). H_o values are closely parallel for 4 of the 5 primers, with the exception being primer OPA2. H_{sp} measures genetic diversity within a species. In this case, since we are examining only one species, H_{sp} is the phenotypic diversity over all individuals examined.

Shannon's index of phenotypic diversity was also used to partition the diversity into within- and between-population components (Table 4). H_{pop} provides a measure of the average diversity within populations. H_{pop}/H_{sp} estimates the proportion of overall diversity

Table 4. Partitioning of the genetic diversity between and within populations of *C. oppositifolia* for five random oligonucleotide primers.

Primer	H_{pop}	H_{sp}	H_{pop}/H_{sp}	$(H_{sp} - H_{pop})/H_{sp}$
OP A2	0.470	0.587	0.800	0.199
OP A11	1.626	2.154	0.754	0.245
OP B1	0.985	1.102	0.894	0.106
OP B8	1.224	1.277	0.958	0.042
OP B17	0.500	0.500	0.999	0.000
Average	0.961	1.124	0.881	0.118

explained by within-population diversity and the proportion of diversity among populations is estimated by $(H_{sp} - H_{pop})/H_{sp}$

The high level of diversity found within populations, H_{pop} , relative to the overall diversity H_{sp} , indicates that most of the diversity (88%) occurs within populations of *C. oppositifolia*. The distribution of variability between and within populations is consistent between primers. All primers indicate that a greater proportion of the overall genetic variation occurs within populations.

Discussion

The ability to detect and monitor genetic variation is essential for devising conservation strategies. The several hundred commercially available RAPD primers provide

the possibility of sampling a large number of genetic markers. This creates ability to detect high levels of polymorphism and to provide a higher degree of resolution often where allozymes may fail to detect such variation (Helenum and Ganders 1985; Brauner et al. 1992; Dawson et al. 1995). However, RAPDs are expressed as dominant markers and cannot be used to estimate allele frequencies from genotype frequencies without certain assumptions (Brauner et al. 1992). RAPDs rely on the assumption of Hardy-Weinberg equilibrium and rely on the frequency of the recessive genotype to estimate allele frequencies, thereby greatly increasing sampling error. Based on this assumption Lynch and Milligan (1994) have recently designed methods to estimate population parameters.

This study used Shannon's index (H_o) to estimate the distribution of genetic diversity within and among populations. Shannon's index is a widely used index in community ecology and is a measure of species diversity (Ludwig and Reynolds 1988). In this study, "species" are replaced by "phenotypes" (RAPD profiles of an individual for a given primer). Dawson et al. (1995) cautions against applying Shannon's index to RAPD data due to the fact that Shannon's index is relatively insensitive to skewing effects caused by the inability to detect heterozygous loci. Another flaw in Shannon's index is that with more bands per primer, more phenotypes will be possible, and by summing a larger number of p_i 's may tend to overestimate the level of diversity. This study also did not analyze the number of shared bands nor generate similarity measures comparing the two populations. One final shortcoming of this study was that only 17 RAPD loci were scored.

These preliminary results still offer useful insight regarding the genetic diversity of *C. oppositifolia*. Most of the diversity was detected within

populations. This is in agreement with Hamrick (1990), who indicated that for woody, outbreeding, perennial plants, most of the variation was found within populations. But in contrast, island endemic species tend to have very low levels of genetic diversity across species boundaries and most likely less diversity within populations (Helenurm and Ganders 1985; Brauner et al. 1992). It should be noted that the three studies to which comparisons have been made are based on allozyme data. Allozymes have been used extensively to provide estimates of genetic variation. Direct comparisons between allozymes and RAPDs are difficult because of the number of factors relating to information content, and the different approaches used to screen, score, and analyze polymorphisms (Dawson et al. 1995).

Overall phenotypic diversity (H_{sp}) for *C. oppositifolia* falls below what was observed for *Gliricidia sepium*, a tropical outbreeding tree species (Chalmers et al. 1992). This tends to suggest that there are fewer, but a more diverse distribution of phenotypes in *C. oppositifolia* populations. Alternatively, the overall

genetic diversity is slightly lower than what is observed, but is structured in a manner that distributes it efficiently. Island endemics are considered to be genetically depauperate and hypothesized to contain only a subset of the genetic complement found in their long distance relatives (Carlquist 1980). However, *C. oppositifolia* has retained a certain level of polymorphism and diversity in its fragmented habitat. I can offer two explanations: (1) fragmented populations are pockets of a larger continuous population that has not experienced the deleterious effects of small population size and number; and (2) *C. oppositifolia* has always been rare and continues to persist with its initial genetic complement.

The two explanations raise interesting questions that will be researched. How does the breeding system of kauila affect the distribution of diversity? Is there any differentiation between populations? If so, how much? Why is *Alphitonia ponderosa* ([Rhamnaceae] also given the name kauila by the Hawaiians because of its similar hard wood and gross morphology) more common and not as

threatened? Is this difference solely due to habitat degradation or is there also a genetic component?

RAPDs have been shown to produce scorable loci (bands) that detect a certain degree of variation in *C. oppositifolia*. RAPDs can be used to assess variation and population differentiation for *C. oppositifolia* and such information be applied to decisions regarding the need for conservation of this rare tree in Hawaiian cultural heritage.

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R. Lani Stemmermann, 1952-1995

Dieter Mueller-Dombois

Kailua, Hawai'i May 15, 1995

This is to acknowledge with great sadness and regret the untimely death of Ruth Leilani Stemmermann, who died at her home in Hilo on March 13, this year. For more than two years, Lani had suffered from malignant lymphoma. She fought her illness courageously by following rigorous schedules of chemotherapy and radiation as prescribed by her doctors. There were several respite periods during her illness, when Lani resumed her teaching, field research, and writing activities. But her immune system finally gave way in the few weeks before her death.

Throughout her illness, Lani maintained a remarkably positive composure. As her former graduate research advisor and subsequent colleague in collaborative research on Hawaiian ecosystems and their vegetation, I am proud of Lani, and humbled to write about her from my perspective.

Lani Stemmermann was born in

Hilo, on September 7, 1952. Her childhood years were spent on the Big Island. She graduated from the Hawai'i School of Girls in 1970 and thereafter attended Pitzer College in Clairmont, California. From there, she graduated with a BA degree in 1974 and then applied for graduate study in botany at the University of Hawai'i.

She received a two-year research assistantship with Prof. C. H. Lamoureux, and in 1976 continued her graduate work as a teaching assistant in the UH Botany Department. Lani received her M.S. degree in Botanical Sciences for anatomical and taxonomic studies of Hawaiian sandalwood (Stemmermann 1977). From her thesis, she published two papers in *Pacific Science* (Stemmermann 1980 a,b).

During her study for the masters degree, Lani took my course in vegetation ecology in the spring of 1975, now 20 years ago. Afterwards, she expressed her desire to

continue her graduate studies for a PhD degree in this area, and then became my graduate student in

1980. In the meantime, she did consulting work for the Army Corps of Engineers on Micronesian wetland vegetation (Stemmermann and Proby 1978, Stemmermann 1981a). In 1980, I received a three-year research award from the National Science Foundation. This provided the main financial support for Lani's PhD work, which focussed on the ecology of *Metrosideros polymorpha*

('ōhi'a lehua) varieties (Stemmermann 1981b, 1983, 1986).

After graduating with a PhD in Botanical Sciences in 1986, Lani

became an instructor and then assistant professor at the University of Hawai'i and Community College

at Hilo where she taught courses in plant ecology, biology, and environmental sciences.

There, Lani pioneered the combination of field instruction with lecture room and laboratory teaching. She was successful in getting support from the University Administration for the purchase of vehicles to take students into the field.



Lani loved field work and she jumped at every opportunity to traverse Hawai'i's vegetation and landscapes. On the Big Island, she became disturbed by the Military's

destructive effects on rare Hawaiian plant assemblages in the Pohakuloa training area. She actively fought for the survival of these rare plant communities by citing the Military for disregard; see Stemmermann vs. Stone (1989) and Stemmermann (1989). Her complaint was initially turned down by the courts, but Lani's courageous and persistent stance turned into success and compliance by the Military, eventually.

In 1990, Lani became a co-principal investigator in a new NSF project on "Ecosystem Dynamics in Hawai'i", a collaborative effort of Stanford University and the University of Hawai'i. Lani's task in this effort was to set up a "Common Garden" for the study of *Metrosideros polymorpha* varieties. This new task grew from Lani's dissertation research, in which she had established a smaller type of "Common Garden" to test whether the "pubescent-leaved" versus "glabrous-leaved" varieties of *Metrosideros polymorpha* would retain their morphological distinctness when grown in a common environment. Indications of "true breeding" of *Metrosideros* varieties

were strong from Lani's dissertation research, but a larger data set, studied over a longer time period, was needed to establish more convincing results.

For this new "Common Garden" study, Lani collected *Metrosideros* seed from five elevational sites (lowland, submontane, montane, upper montane, subalpine) and two parallel running lava flows, a young and an old flow, along the Saddle Road. At each of the 10 sites, she searched for pubescent and glabrous *Metrosideros* varieties. In total, she found 15 parental populations from which she collected and planted seeds over a three-year period. It took many field trips to catch the ripening capsules at exactly the right time of opening to collect their seeds for the experiment. Many times, seed lots were reduced to only a few viable seeds after collecting, and many germinants perished after emergence in spite of great care in nursing them to seedling stage.

When Lani's illness began to force her into reduced activities, in June 1993, she had established about 500 seedlings of both varieties from the

five elevational sites, with the help of her able technical assistant and close friend, Zoe Thorne.

At that time, the task of continuing the “Common Garden” experiment, fell upon me. In the following two years, we were able to complement the “Stemmermann Common Garden” of *Metrosideros* varieties more or less according to Lani’s plan. Currently, a new graduate student in botany at UH (Susan Cordell-Hart) is completing a Master’s degree with a study of the physiological differentiation of *Metrosideros polymorpha* varieties, using Lani’s “Common Garden” seedlings and their parental field populations. Several other investigations can, and should be, carried out. An in-depth anatomical study is planned by Dr. David Webb of UH. The shade tolerance and light requirements should be studied among the varieties, the glabrous form likely to display increased shade-tolerance relative to the pubescent pioneer form of *Metrosideros*. Differences in nutritional requirements and water efficiency should be clarified, and the genetic differentiation of the varieties deserves particular atten-

tion, as well as their morphological flexibility and reproductive phenology; just to mention a few important ecological questions that can now be answered through new research, based on Lani’s pioneering work.

Lani did not get much credit, so far, from her three years of countless hours spent in establishing this new research facility. For her, this was primarily a service task, often combined with drudgery and hard work. However, with this “Common Garden”, she left a living legacy in Hawai‘i that calls for further use in research application and expansion by collecting and growing *Metrosideros polymorpha*, our dominant native rain forest tree, from all islands and habitats (including the bogs with dwarf varieties), for gaining an improved understanding of the adaptative capacities of this Hawaiian keystone species. A follow-up of her work is needed, and as Lani would have put it: *Holomua ka no‘eau*.

A recent memorial for celebrating Lani’s life was arranged at the Lyon Arboretum in Honolulu, where, at this occasion, several varieties of

Metrosideros were planted in her honor. Just before her death, we could report to Lani that one of her "Common Garden" seedlings had set flower. She was delighted, and she guessed correctly that it was a pubescent-leaved variety of *Metrosideros* from the montane environment.

In Lani Stemmermann, I have lost one of my most precious graduate students. Her death came much too soon. All those who knew Lani, were privileged to have made her acquaintance, and many of us were enriched by having had her as a friend and colleague. Lani Stemmermann certainly has advanced the botanical sciences in Hawai'i with her strong focus on conservation biology and applications to conservation management and policy. For this, she will be remembered as a foremost Hawaiian biologist.

Following is a selection of her publications, which provides an insight into her professional track record. It further points to the loss of potential "know-how" that Lani would surely have contributed, had her life not been terminated so

harshly at the young age of only 42 years.

Selected publications of Dr. Lani Stemmermann (put in chronological order):

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Minutes of the Hawaiian Botanical Society

February 6, 1995

The meeting was called to order by President Camille Barr at 7:50 p.m. She asked if there were any visitors to be introduced.

Minutes from the January meeting were read by Leilani Pyle and accepted.

Treasurer Ron Fenstemacher had no report at this time. However he voiced a concern about monies received from the three life memberships we received in 1994, in the amount of \$450, that those need to be set aside in an escrow account and not counted in current operating expenses. However, without that income last year and donations, we would have been in the red. Therefore Ron strongly recommended that we double dues for 1996. President Camille Barr asked that the members please consider this option.

Membership report. Vicky Cara-

way announced that we had a new member, Gerald Toyama, who was in the audience and was recognized. Vicky also announced that Life Member Peter Connally, of Haleakalā National Park, passed away last year. Don Gowing also announced that our oldest member in number of years membership, Clinton Wells, of Kapaʻa, passed away.

Old Business

Membership Drive: Camille reminded us that February was Membership Drive Month and there will be a special plant donated by Alvin Yoshinaga for each person that brings in a new member. The member that brings in the most members will get a book.

New Business

Camille announced that the Botanical Society used to give away a prize to an outstanding botany undergraduate student and she recommended we do that again. She initiated a committee to do this and got two volunteers to join her committee: Orlo Steele and Robert Pattison.

Camille proposed a way to make

money for the society — tee shirts. Her mother Louise is designing a tee shirt and other design suggestions are welcome. Other suggestions were a tote bag, too.

Benton Pang announced that in the past we have donated to Paradise Pursuits, a Hawai'i Audubon sponsored local TV quiz show on natural history and that in the past we have given a prize.

Plant and Speaker of the Month

Rick Palmer gave a presentation on the rare and endangered *Cyanea maceldowneyi*, named after George Maceldowney of the Wahiawā Botanical Gardens. There are only 75 left in the wild on Maui.

The main speaker, Dr. William Klein, Executive Director of the National Tropical Botanical Garden, was introduced by Lisa Stratton. His topic was National Tropical Botanical Garden in the 20th Century. NTBG is a national, privately supported tropical botanical garden chartered by Congress in 1964. It has four kinds of activities:

1. To collect and curate living plants.
2. Obtain all existing

3. knowledge about each plant.
4. Nurturing the plants and learning their individual requirements.
5. Sharing the knowledge through classes and tours.

He showed us plans for a new proposed entrance to the Allerton Gardens and the Lawai Gardens and plans for the layout of the gardens in relation to the entrance.

He also told us of plans for their other gardens in Hana, Maui, and the Fairchild Gardens in southern Florida. Dr. Klein answered questions and the meeting was adjourned and refreshments were served.

March 6, 1995

The meeting was called to order by President Camille Barr at 7:45 p.m. She asked if there were any visitors to be introduced.

Minutes from the February meeting were read by Leilani Pyle and accepted as read.

Treasurer Ron Fenstemacher gave a report for February. There was income of \$305.55 and expenses of \$371.23. Our balance on hand is \$4,539.40.

Membership Report. Vicky Caraway announced that we had 18 new members and read their names for approval by the membership. This brings our total membership to 197.

Old Business

Membership Drive: Members who brought in new members were given a native plant grown by Alvin Yoshinaga, membership chairman. The deadline for the membership drive has been extended to March 28 and the person who brings in the most members will receive a book, *Hawai'i's Plants and Animals* by Charles Stone and Linda Pratt with illustrations by Joan Yoshioka.

Tee Shirts: Camille showed us the design created by Louise Barr for a Hawaiian Botanical Society tee shirt, an artistic drawing of *Brighamia*. A discussion followed about adding flowers and/or a stem. Camille will go back to the artist and see how it can be artistically arranged.

New Business

Fish and Wildlife Service:

Camille gave an update on the work she is doing on behalf of the Soci-

ety as part of a working group on endangered species with the US Fish and Wildlife Service.

Wildlife Poster: Steve Montgomery representing the Hawaii Conservation Council asked for a donation of \$300 to help with the production of a local poster by a local artist in honor of National Wildlife Week. A motion was made to refer the request of \$300 to our special Neal-Miller Trust Fund of which Dan Palmer is one of the trustees. It was seconded and passed by the membership.

Plant and Speaker of the Month

Tim Motley, UH graduate student, gave a presentation entitled, "A quick Trip Through the Philip-pines."

The main speaker, Dr. Warren "Herb" Wagner, Jr. of the University of Michigan, gave his presentation, "Update on Hawaiian Ferns."

April 3, 1995

The meeting was called to order by President Camille Barr at 7:35 p.m. She asked if there were any visitors to be introduced.

Minutes of the March meeting were read by Leilani Pyle. Lorin Gill corrected the name of the organization, Conservation Council for Hawai'i

Treasurer Ron Fenstemacher gave a report for March. There was income of \$90 plus interest and expenses of \$411.54. Our balance on hand is \$4,186.34 plus interest.

Membership Report. Alvin Yoshinaga announced that we had 10 new members and read their names for approval by the membership. This brings our total membership to 207.

Old Business

Membership Drive: Members who brought in new members were given a native plant grown by Alvin Yoshinaga, membership chairman.

The winner of the Membership Drive was Gerald Toyomura. We was presented with the book *Hawai'i's Plants and Animals* by Charles Stone and Linda Pratt with illustrations by Joan Yoshioka. **Tee Shirts:** Camille showed us the new Botanical Society tee shirts. She has the supply and they sell for \$12 a piece.

New Business

Earth Day is April 23 at Kapi'olani Park. The Hawaiian Botanical Society will have a table there and Camille asked for volunteers to help. **May Meeting:** The president reminded us that at the May meeting we have a plant raffle and urged members to donate plants for this meeting. It will be the last meeting before the summer break.

Prizes for the Science Fair: The Science Fair is this coming Thursday and the president announced that there would be prizes given by the Hawaiian Botanical Society.

Announcements: Alvin Yoshinaga announced that there would be an O'ahu memorial service for Lani Stemmerman at the Lyon Arboretum, 2:30 on Sunday, April 23.

Pat Conant announced that there would be a Sierra Club Work Group to pull *Miconia* in the back of Kalihi Valley on April 16. He invited our members to participate. They are meeting at the Church of the Crossroads at 8 a.m. Camille Barr asked that we help the treasurer by putting a note on all funds given to him (especially in cash)

stating what the funds are for and the name of the person they came from.

Plant and Speaker of the Month

Rob Anderson, UH botany student,

gave a presentation entitled, "Rare and Endangered Plants on O'ahu."

The main speaker was Bill Garnett. He gave his presentation "Outplanting Activities on O'ahu: How do we Stand?"

Science Fair Committee Report

May 1, 1995

The Science Fair committee distributed the following awards at the 38th Hawai'i State Science and Engineering Fair. We'd like to thank Char & Associates for donating the botany tee shirts and second place prizes for each division.

First Place in the Senior Division

Leroy Slingerland and Kekoa Renaud from Kapa'a High School

Determining the Tolerance of
Chironomus hawaiiensis and
Procambarus clarkii to CuSO_4
Treatments Used to Control
Pomacea canaliculata in

Hawai'i's Lowland Taro Fields

They received a \$50 check, and each student got an autographed copy of *La'au Hawai'i*, a botany department tee shirt, and a one-year membership to the Hawaiian Botanical Society.

Second Place in the Senior Division

Jenny Sakuoka from Leilehua H.S.

Identifying the Subspecies of
Acacia koa Through Growth
Morphology

Jenny received a \$25 check, an

autographed copy of *La 'au Hawai 'i*, a botany department tee shirt and a one-year membership in the Hawaiian Botanical Society.

First Place in the Junior Division

Kauka Case of Waimea Elementary and Intermediate School

'Ulu Sap-Stronger and Strongest

Kauka received a \$50 check, an autographed copy of *La 'au Hawai 'i*, a botany department tee shirt, and a one-year membership to the Hawaiian Botanical Society.

Second Place in the Junior Division

Shannon Gascon of Wahiawa Intermediate School

Invasion Hawai'i: The Prevalence of Alien Species on the Kaulana Trail

Shannon received a \$25 check, an autographed copy of *La 'au Hawai 'i*, a botany department tee shirt and a one-year membership in the Hawaiian Botanical Society.

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LETTER OF THE HAWAIIAN BOTANICAL SOCIETY
DEPARTMENT OF BOTANY
UNIVERSITY OF HAWAII AT MĀNOA
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